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16. ABSTRACT

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Comparison of Laboratory and Field Avoidance Behavior of Fish in Heated Chlorinated Water

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Abstract

The effects of intermittent chlorination and temperature selection on the movement of fish were studied in an integrated field and laboratory project on the New River at the Glen Lyn Power Plant in southwestern Virginia. Over a temperature range of 7–36 C, the total number of fish sampled from the intermittently chlorinated thermal effluent was lower than control values ($P \leq 0.09$) when total residual chlorine (TRC) concentrations were ≥0.15 mg/liter. After seasonal variations were segregated into discrete intervals of field temperature and fish avoidance of TRC, a decline in fish abundance in the chlorinated, heated discharge was observed within 95% confidence limits. In summer field temperatures of 27–30 C, fish avoided a TRC concentration that ranged from (95% confidence limits) 0.19 to 0.28 mg/liter TRC; when temperatures were falling from 26 to 7 C, they avoided 0.23–0.42 mg/liter TRC. The two most consistently sampled fish species, spotfin shiner *Notropis spilopterus* and whitetail shiner *N. galacturus*, generally avoided areas of the discharge when TRC concentrations were equal to 0.18 mg/liter in the temperature range of 24–29 C. At field temperatures of 30–34 C, however, whitetail shiners avoided 0.04 mg/liter TRC; spotfin shiner numbers did not decline until TRC concentrations were above 0.12 mg/liter. The lowest laboratory concentration avoided by spotfin shiners was 0.20 mg/liter TRC. The whitetail shiner was more sensitive in the laboratory at 12, 18, and 24 C (avoiding 0.11 mg/liter TRC) than at 30 C (0.20 mg/liter). Selection of test water heated to the preferred temperature of either species prior to chlorine addition did not alter subsequent avoidance of TRC concentrations except for spotfin shiners tested in combined residual chlorine (CRC) concentrations at 12 C (0.38 mg/liter CRC avoided). In most cases, laboratory-determined avoidance concentrations predicted accurately the TRC concentrations that would elicit the avoidance behavior of fish under natural field conditions. Based on published acute toxicity tests with intermittent chlorination, spotfin shiners and most other fish species generally avoided chlorine residuals 50% or less of the median lethal concentrations.

Fish are capable of avoiding sublethal concentrations of chlorine under field (Cherry$_2$ et al. 1977; Grieve et al. 1978) and laboratory conditions (Fava and Tsai 1976, 1978; Cherry$_2$ et al. 1977; Cherry$_3$ et al. 1977; Bogardus et al. 1978; Cherry et al. 1978, 1979; Larrick et al. 1978). Laboratory avoidance responses of fish to intermittent chlorination, however, have not been verified with extensive field sampling as part of a single study (see reviews by Brungs 1973, 1976). The objectives of our research were to: (1) determine the abilities of fish to avoid intermittent chlorine discharges under field conditions; (2) establish laboratory avoidance concentrations with and without attraction into heated water as an influential factor; and (3) evaluate the validity of using laboratory-derived data to predict concentrations that cause avoidance responses in the field.

Present National Pollution Discharge Elimination System (NPDES) limitations for intermittent chlorination in Virginia are 0.50 and 0.20 mg/liter free residual chlorine (FRC) for maximum and mean weekly discharges, respectively. Although discharges of the Glen Lyn plant (New River, southwestern Virginia) have

contained total residual chlorine (TRC) concentrations as high as 0.62 mg/liter and FRC concentrations of 0.58 mg/liter, no fish kills have been observed in this area (Cherry$_2$ et al. 1977). This suggests that even though toxic concentrations are exceeded for some species, resident fish manage to avoid th[illegible].

Methods

The Glen Lyn plant is a coal-fired 400-megawatt electric generating station with a once-through cooling system that is intermittently chlorinated (three times daily) for removal of nuisance organisms in the condenser tubes. Water is pumped from the New River, passed through the plant, and returned to the environment via two routes: (1) directly into the New River; and (2) through a 200-m discharge canal that empties into the East River, a tributary of the New River (Fig. 1). Field sampling was carried out at two similar sites in the East River and one in the New River. Site 1, directly below the confluence with the discharge canal, had a sand-cobble substrate and varied in depth from 0.3 to 1.5 m (near shore to midstream). Heated water (8–12 C above ambient) was discharged from the canal into the East River at about 7.6 m^3/second. During chlorination, this area received chlorinated effluent for 30–40 minutes; peak TRC concentrations were 0.50 mg/liter. Site 2 was 25–30 m downstream and had 10–20% lower peak chlorine concentrations. Site 3 was located within the thermal effluent of the New River but generally was beyond the influence of chlorine (Cherry$_2$ et al. 1977). The substrate was composed of sand, cobble, and boulders, and depths ranged from 0.2 to 2.1 m.

Field Sampling

The normal procedure for chlorinating the water-cooling system was varied on the morning of each sampling day so that chlorine gas was applied until one of five possible FRC concentrations (0.1, 0.2, 0.3, 0.4, 0.5 mg/liter) was measured in the plant. The schedule determining the particular dose applied on each day was randomized to prevent sampling bias, and each week samples were taken on one control (0.0 mg/liter TRC) and two dosing days. Following chlorination of the condenser water, TRC and FRC concentrations were measured every 5 minutes at Site 1 by amperometric titration.

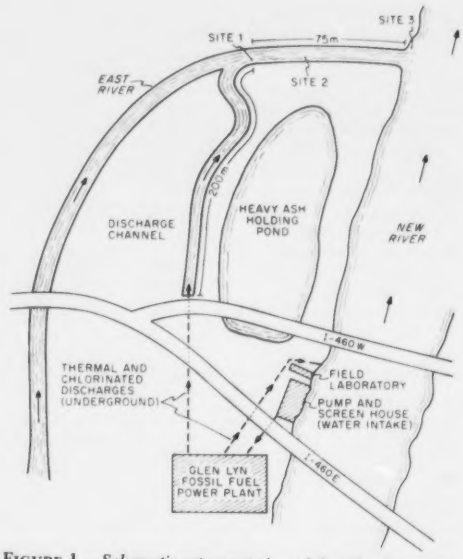

FIGURE 1.—*Schematic representation of the Glen Lyn area, Virginia, showing the relationship of the field sampling sites to the power plant and field laboratory.*

Attempts to measure chlorine at Site 2 were not made with each sampling effort but enough samples were taken to establish the relationship between peak TRC concentrations at Sites 1 and 2. Temperature, pH, dissolved oxygen, and flow rates were measured at all field sites with each sampling effort; nitrates, ortho- and total phosphates, sulfates, and ammonia were measured monthly (Table 1).

River areas approximately 12 × 12 m were sampled with a 9-m seine having a 2-m bag depth and either 10- or 13-mm stretch mesh. Each area was seined twice from upstream to downstream and samples were collected consecutively from sites 1 to 3 within 40 minutes after the peak TRC concentration was measured at Site 1. Site 1 was sampled in 1977, and all three sites were sampled in 1978. During the first 4 months of sampling, all fish were preserved in 10% formalin and later identified; thereafter, all fish were identified and counted in the field and immediately returned to the river.

The Wilcoxon rank sum test was used to compare differences in the number of fish sampled between control days, low-dose days (TRC < 0.15 mg/liter), and high-dose days (TRC ≥ 0.15 mg/liter). This was done by com-

TABLE 1.—*Water chemistry variables measured in the East River, southwest Virginia, during 1977 and 1978.*

Variable	Average	Range
Alkalinity (mg/liter as $CaCO_3$)	52.90	39–54
Hardness (mg/liter as $CaCO_3$)	68.30	51–76
Conductivity (μmhos/cm)	119.40	90–155
pH	7.80	7.3–8.2
Nitrate-N (mg/liter)	5.77	2.04–6.10
Ammonia-N (mg/liter)	0.08	0.005–0.09
Total phosphate (mg/liter)	0.07	0.05–0.20
Dissolved orthophosphate (mg/liter)	0.03	0.005–0.04
Sulfate-S (mg/liter)	13.80	10.0–34.6
Dissolved oxygen (mg/liter)	9.50	5.0–14.0

bining the data from the two groups being compared and ranking (from lowest to highest) the numbers of fish collected under each set of conditions. The ranks were then separated into their respective groups and summed (T) for the group with the smallest sample size. When T was extremely small (or large), then the null hypothesis that the two populations were identical was rejected (Conover 1971). Levels of significance were determined using the normal distribution. Data for sites 1 and 2 were pooled according to season (summer or fall) for 1977 and 1978 to determine statistically significant ($P \leq 0.05$) field avoidance concentrations: those TRC concentrations at which there were significant decreases in the abundance of fish compared to control samples.

The principal inorganic constituents of the TRC are free residual chlorine (FRC), composed of hypochlorous acid (HOCl) and hypochlorite ion (OCl^-), and combined residual chlorine (CRC), predominantly monochloramine (NH_2Cl) in the New River. Combined residual chlorine and FRC (particularly the HOCl fraction) exert different effects on the avoidance responses of fish, which may influence the different toxicities of these two components (Fava and Tsai 1976; Cherry$_3$ et al. 1977; Heath 1977; Larrick et al. 1978). In the present study detectable FRC concentrations existed in the discharge, but were not present with every sampling effort and usually comprised less than 20% of the TRC. Therefore, all field and laboratory avoidance thresholds were expressed in terms of TRC.

Laboratory Avoidance

The methods and equipment used for determining laboratory avoidance thresholds have been previously described by Cherry$_2$ et al. (1977), Cherry$_3$ et al. (1977), Cherry et al. (1978), and Larrick et al. (1978). All avoidance trials were carried out under semicontrolled laboratory conditions in a trailer-type laboratory located adjacent to the New River. River water was pumped directly into the laboratory for use in all holding tanks and avoidance tests, so that fish were exposed to the same daily and seasonal variations in water quality encountered in the river.

During the avoidance tests, water was pumped from two 64-liter, temperature-controlled, water baths into two parallel troughs. Water entered the two troughs from both ends and drained from the centers, which provided a sharp vertical boundary between chlorinated and clean water. Fish were placed in the troughs and allowed 1 to 3 hours to adjust to the experimental conditions. Observations were made from a closed-circuit television monitoring system. Water samples for determination of TRC and FRC concentrations by amperometric titration were taken from the troughs by gravity flow through tygon tubes (Cherry$_3$ et al. 1977).

After the adjustment period, fish were observed for 10 minutes (control period) to determine the relative time spent in each half of a trough. To determine avoidance responses, a chlorine solution (calcium hypochlorite $Ca(OCl)_2$) was added to one water bath so that successive doubling increments of 0.025, 0.05, 0.10, 0.20, and 0.40 mg/liter TRC were obtained in one half of each trough. Stock TRC solution was also pumped into the water bath to maintain a relatively constant chlorine concentration. When each target concentration was reached, fish were timed for 10 minutes; then the chlorine concentration was raised to the next target level. Total residual chlorine tests were conducted at 12, 18, 24, and 30 C acclimation temperatures in the manner described above with 20–80% of the TRC in the FRC form (Table 2). The CRC trials for fish avoidance were conducted with stock solutions of greater than 95% monochloramine (NH_2Cl). Quantities of ammonium chloride (NH_4Cl) were added to the TRC stock solution to create a predominantly monochloramine solution at ≥7.9 pH. Application of ammonia concentrations did not contribute directly to avoidance behavior in the preliminary investigations or in work by Cherry et al. (1978). Tests were carried

TABLE 2.—*Types of chlorine avoidance trials used for spotfin and whitetail shiners to determine the first significant avoidance concentration from exposures of total residual chlorine (TRC) and combined residual chlorine (CRC as ≥95% monochloramine) during temperature conditions with and without preferred temperature influence. FRC is free residual chlorine.*

Type of avoidance trial	Major component present	Fish acclimation temperature tested (C)
TRC[a]	20–80% CRC and FRC to 80–20% CRC and FRC depending upon river water quality	12, 18, 24, 30
CRC[b]	≥95% monochloramine comprising the TRC	12, 24
TRC in preferred temperature[a,c]	20–80% CRC and FRC to 80–20% CRC and FRC depending upon river water quality	12, 24
CRC in preferred temperature[b,c]	≥95% monochloramine comprising the TRC	12, 24

[a] The TRC trials consisted of a certain proportion of CRC and FRC (with HOCl content in the FRC) carried out during clear New River conditions.

[b] The CRC dosing arrangement (target concentrations) was the same as that for TRC. The CRC trials were different in that they were carried out during river conditions of high flow when FRC was minimal. Stock dosing solutions in the laboratory were also seeded with NH_4Cl to remove any remaining FRC.

[c] At specified acclimation temperatures (12 and 24 C), fish first were attracted into water of the trough that was heated to their preference temperature; then water was chlorinated with TRC or CRC.

out at acclimation temperatures of 12 and 24 C. Preferred-temperature tests also were conducted at 12 and 24 C acclimation temperatures. After the initial 10-minute control observation period, the temperature in one half of each trough was raised to the temperature preferred by the species. For this study, both spotfin and whitetail shiners preferred about 27 C when acclimated to 24 C; half the trough, therefore, was raised to 27 C and the other half kept at 24 C. After this temperature differential was established and attraction occurred, fish were timed for 10 minutes to document a preference for the warmer water, then exposed to TRC or CRC as outlined above.

The first avoidance concentration was that concentration first causing a significant decline in the residence time of the fish on the chlorinated side of the trough compared to the control periods. Eight replicates per species were run for each test and acclimation temperature. Significant differences ($P \leq 0.05$) between the mean residence times of the eight replicates at each chlorine dose level were determined by Duncan's New Multiple Range Test (Barr et al. 1976).

The temperature preferences of whitetail shiners were established in the same manner as reported earlier for the spotfin shiner, by the procedures of Cherry, et al. (1977). These results were incorporated into the trials for determining the influence of temperature preference upon the avoidance of TRC and CRC when fish were acclimated at 24 C. Tests were conducted in an epoxy-painted, stainless-steel trough with 12 250-watt infrared lamps underneath. These lamps heated the test water to various temperatures, producing a gradient ranging from cold to warm extremes. Eight fish were used for each test and each test was duplicated at acclimation temperatures of 6, 12, 18, 24, and 30 C. After an initial adjustment period (60 minutes minimum), observations were made on the positions of the fish within the gradient at 2-minute intervals for 40 minutes. The mean temperature then was calculated from these observations and was considered to be the selected temperature. The final thermal preferendum was then estimated from a graph of selected versus acclimation temperatures (Cherry, et al. 1977).

Results

Field Avoidance

A statistically significant decrease ($P \leq 0.02$) from control levels in fish abundance was observed during the summer (average discharge temperature, 31 C) when the TRC was 0.15 mg/liter or greater (Table 3). Over the range of field concentrations, 0.15–0.36 mg/liter TRC, for which this reduction was evident, the 95% confidence interval for the threshold avoidance concentration was 0.19–0.28 mg/liter TRC. A similar reduction in fish abundance was noted in the fall (average discharge temperature, 18

TABLE 3.—*Number of fish collected, relative abundance of the major species, and the peak total residual chlorine (TRC) concentrations for control, low-dose (TRC < 0.15 mg/liter), and high-dose (TRC ≥ 0.15 mg/liter) samples from the East River, Virginia. Data for sites 1 and 2 were pooled according to season; samples were collected during 1977 and 1978. Levels of significance (P) were determined with the Wilcoxon rank sum test by comparing the low-dose and high-dose samples with controls.*

Season	Treatment	N	Number of fish			Peak TRC concentration (mg/liter)			
			Mean	SD	Range	Mean	SD	Range	P
Summer (27–36 C)	Control[a]	15	25	34	0–133				
	Low dose	15	28	33	0–127	0.10	0.03	0.04–0.14	0.50
	High dose	10	3	4	0–14	0.24	0.08	0.15–0.36	0.02
Fall[b] (<25 C)	Control[a]	11	17	23	0–78				
	High dose	12	5	6	0–21	0.32	0.15	0.20–0.71	0.09

Species	*Relative abundance (%)*
Notropis spilopterus (spotfin shiner)	43.6
Campostoma anomalum (central stoneroller)	13.2
N. galacturus (whitetail shiner)	11.3
N. volucellus (mimic shiner)	8.8
N. rubellus (rosyface shiner)	8.2
N. telescopus (telescope shiner)	5.0
N. stramineus (sand shiner)	2.1
Pimephales notatus (bluntnose minnow)	2.1
N. photogenis (silver shiner)	1.3
N. chrysocephalus (striped shiner)	1.2
Micropterus dolomieui (smallmouth bass)	1.0
All other species	2.2

[a] No residual chlorine was present on control days.
[b] No peak TRC values ≥0.15 mg/liter were measured.

C), but the decline was not as significant ($P \leq 0.09$); avoidance concentration was 0.32 mg/liter TRC (95% confidence interval, 0.23–0.42). Eleven species contributed most of the samples (Table 3). Although the spotfin shiner and the central stoneroller were the most abundant species collected from the discharge area, the whitetail shiner was more consistently sampled than the central stoneroller on a daily basis. Therefore, spotfin and whitetail shiners were used in the evaluation of effects of chlorine upon avoidance behavior. Neither species generally was found in high numbers during the fall months, so only summer abundance is considered. Within the temperature range 24–29 C, no apparent differences in the responses between spotfin and whitetail shiners were evident; both species decreased in abundance at TRC concentrations greater than 0.18 mg/liter. Within the temperature range 30–34 C, however, spotfin shiner abundance first declined at TRC concentrations of approximately 0.12 mg/liter, and that of the whitetail shiner at 0.04 mg/liter (Fig. 2).

Laboratory Avoidance

Spotfin shiners acclimated to either 24 or 30 C first avoided TRC at 0.12 mg/liter (Table 4). The lowest TRC concentrations avoided by whitetail shiners were 0.11 mg/liter for fish acclimated to 24 C, and 0.22 mg/liter for those acclimated to 30 C. Although both fish species were infrequently sampled at field temperatures <18 C, laboratory avoidance at acclimation temperatures ≤18 C were 0.21 and 0.11 mg/liter for the spotfin and whitetail shiners, respectively. In preferred-temperature tests, both species were strongly attracted to the side of the troughs heated to 27 C when acclimated to 24 C. Spotfin and whitetail shiners increased their residence time over control values by ~50%, but this selection of heated water did not alter the TRC concentrations subsequently avoided. The TRC threshold avoidance concentrations were 0.20 and 0.12 mg/liter for the spotfin and whitetail shiners, respectively. Attraction into water heated to the preferred temperature (21 and 17 C, respectively, for spotfin and whitetail shiners) was even greater when

Errata

Please make the following corrections on page 530 of this year's *Transactions* (Volume 110, Number 4, July 1981).

Table 3, footnote b: change "≥15" to "<15" mg/liter.

Right text column, 2nd line under the subhead: change "0.12" to "0.21" mg/liter.

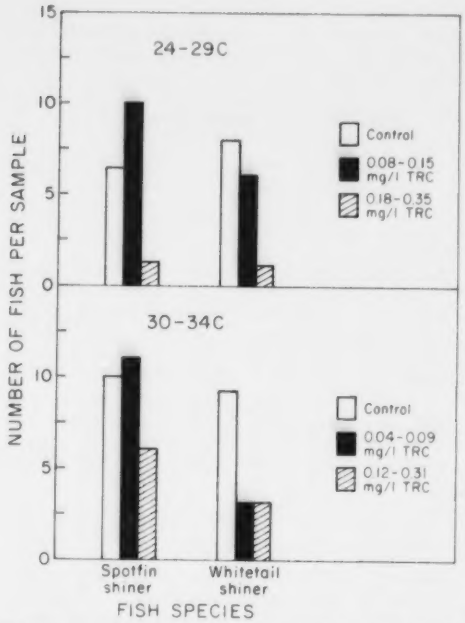

FIGURE 2.—*Mean abundances of spotfin and whitetail shiners in the field for various total residual chlorine (TRC) concentrations and temperature ranges of 24–29 C and 30–34 C during 1977–1978.*

fish were acclimated to 12 C. The TRC avoidance concentration was the same at either acclimation temperature for the spotfin shiner, although whitetail shiners avoided a higher concentration (0.21 mg/liter) at 12 C than at 24 C (0.12 mg/liter).

Avoidance of CRC (mainly as monochloramine) was consistent for spotfin and whitetail shiners tested at 12 and 24 C without the influence of temperature selection. Both species first avoided the same target concentration of 0.20 mg/liter (actual doses varied from 0.20 to 0.22 mg/liter) (Table 5). Following CRC treatment in the preferred-temperature region of the avoidance troughs, the spotfin shiner avoided a greater concentration when tested at 12 C (0.38 mg/liter) than at 24 C (0.20 mg/liter). Temperature selection did not influence the avoidance response of the whitetail shiner to CRC at either 12 or 24 C; avoidance first occurred at 0.20 mg/liter.

Both fish species appeared to detect TRC sooner than CRC in chlorination trials involving both unheated and preferred-temperature treatments (Table 5). In no case did either fish species avoid a concentration of CRC that was lower than that of TRC. At several acclimation temperatures and temperature preference-chlorination interactions, however, a greater concentration of CRC was needed to stimulate avoidance. For example, in trials not offering a preferred temperature, the whitetail shiner first avoided a CRC concentration twice as high as that of TRC. In this study, the highest concentration first avoided (0.38 mg/liter) occurred in the CRC trials with spotfin shiners influenced by temperature selection for the spotfin shiner; the same species avoided TRC at half this concentration.

The influence of hypochlorous acid, part of the free residuals in the TRC trials, was nearly the same at the first avoidance concentration for each acclimation temperature (Table 5). The HOCl concentration calculated for the first significant avoidance ranged from 0.02 to 0.05 mg/liter and 0.01 to 0.02 mg/liter, respectively, for spotfin and whitetail shiners in trials without the influence of temperature selection.

Discussion

The average summer (24–30 C) TRC threshold at which fish avoided chlorinated effluent in the East River was 0.24 mg/liter. The 95% confidence interval around this mean (0.19 to 0.28 mg/liter) embraced most of the laboratory avoidance data for spotfin and whitetail shiners. We have tested in the laboratory other species that commonly are found in the East River and some which are prevalent in other areas of the country. A comparison of these results and those of other authors indicated that most of these avoidance concentrations overlapped with our field confidence interval (Table 6). The few species of salmonids that consistently avoided concentrations less than these confidence limits were not sampled in the field sites of this study. Thus, we concur with Sprague and Drury (1969) that laboratory avoidance tests are accurate predictors of fish avoidance responses to chlorine in the field.

The differences in sensitivity to TRC observed between the spotfin and whitetail shiners under different thermal regimes in the river seemed to indicate that spotfin shiners were better adapted to combined stresses of high temperature and residual chlorine than white-

TABLE 4.—*Laboratory responses of spotfin and whitetail shiners to temperature and concentrations of total residual chlorine (TRC) or combined residual chlorine (CRC as monochloramine). Fish were free to move between chlorinated and unchlorinated water in the same trough. Asterisks (*) denote the first significantly lower residence times from the previous lower target concentrations (Duncan's New Multiple Range Test, P ≤ 0.05).*

Chlorine constituent tested	Acclimation temperature (C)	Test temperature (C)	Percent fish residence time (SD) in target TRC concentrations (mg/liter) of									
			0									
			Acclimation temperature	Preferred temperature	0.02		0.05		0.10		0.20		0.40	
Spotfin shiner												
TRC	12	12	48.3	(7.2)	[a]	44.2	(10.8)	43.8	(8.4)	46.8	(15.0)	23.5*	(9.6)	25.1	(12.2)
	12	21[b]	49.3	(9.8)	76.8	(10.3)	64.7	(20.5)	58.3	(18.0)	47.2	(17.4)	15.9*	(12.3)	16.2	(13.6)
	18	18	52.4	(10.1)	[a]	63.4	(18.1)	54.6	(14.9)	45.3	(13.0)	12.4*	(8.8)	20.3	(2.2)
	24	24	46.3	(7.2)	[a]	44.1	(7.8)	44.8	(9.4)	48.6	(17.0)	21.4*	(8.8)	27.1	(14.7)
	24	27[b]	48.1	(7.4)	69.6	(7.5)	73.8	(38.1)	58.6	(12.4)	42.2	(8.5)	13.9*	(10.7)	23.9	(14.3)
	30	30	55.5	(6.4)	[a]	57.7	(10.3)	48.3	(24.9)	54.1	(14.4)	28.2*	(13.2)	10.0	(8.7)
CRC	12	12	55.6	(6.8)	[a]	50.4	(13.7)	47.7	(13.0)	43.0	(15.8)	22.4*	(9.0)	30.2	(11.2)
	12	21[b]	45.9	(9.7)	71.2	(9.6)	57.2	(20.3)	57.2	(19.3)	44.4	(16.9)	37.9	(13.7)	20.8*	(15.7)
	24	24	54.1	(10.2)	[a]	43.3	(8.2)	47.1	(12.4)	46.4	(11.0)	24.8*	(11.7)	28.4	(14.2)
	24	27[b]	49.4	(7.7)	68.1	(6.5)	69.7	(18.9)	53.9	(17.4)	48.7	(11.6)	15.5*	(11.2)	16.8	(15.3)
Whitetail shiner												
TRC	12	12	47.7	(8.3)	[a]	48.1	(11.9)	47.9	(15.2)	12.7*	(12.0)	12.8	(11.4)	11.7	(10.7)
	12	17[c]	51.0	(11.1)	72.9	(6.8)	54.3	(12.2)	54.2	(16.2)	38.4	(18.3)	17.8*	(9.7)	13.0	(14.5)
	18	18	48.6	(9.4)	[a]	44.6	(9.1)	48.0	(11.3)	13.6*	(13.1)	10.2	(13.0)	5.8	(7.6)
	24	24	46.8	(7.9)	[a]	52.8	(13.8)	63.7	(14.7)	17.9*	(8.3)	9.0	(9.2)	2.2	(1.6)
	24	27[c]	46.2	(5.7)	65.6	(14.9)	55.7	(8.4)	48.9	(16.4)	14.3*	(6.4)	0.4	(0.6)	0.3	(0.3)
	30	30	51.0	(9.3)	[a]	56.9	(17.8)	61.1	(17.1)	52.3	(18.2)	10.4*	(8.6)	5.0	(7.6)
CRC	12	12	47.3	(9.8)	[a]	50.6	(12.2)	50.3	(11.1)	38.4	(13.8)	21.5*	(12.1)	15.2	(9.1)
	12	17[c]	50.1	(8.7)	70.0	(8.3)	56.7	(13.6)	49.1	(18.7)	36.1	(14.1)	19.1*	(10.1)	19.4	(16.4)
	24	24	49.2	(10.0)	[a]	49.6	(14.3)	56.6	(18.4)	52.3	(11.6)	18.8*	(8.9)	17.9	(5.4)
	24	27[c]	49.6	(7.4)	67.3	(12.0)	56.6	(21.4)	59.1	(16.6)	30.8	(17.5)	7.0*	(6.5)	6.4	(12.0)

[a] Avoidance of TRC or CRC evaluated without temperature preference being a factor.

[b] Temperature preferred by spotfin shiners when acclimated at either 12 or 24 C; final temperature preferendum is 24.9–31.9 C (Cherry, et al. 1977).

[c] Temperature preferred by whitetail shiners when acclimated at either 12 or 24 C; final temperature preferendum is 27.2–29.9 C (this study).

TABLE 5.—*Laboratory avoidance responses of the spotfin and whitetail shiners acclimated and tested at 12, 18, 24, or 30 C and exposed to total residual chlorine (TRC) and combined residual chlorine (CRC, ≥95% monochloramine) in trials with and without the influence of temperature preference. Species of chlorine in the TRC trials, free residual chlorine (FRC) and hypochlorous acid (HOCl), are compared at concentrations just prior to and at the first significant avoidance.*

Acclimation temperature (C)	Trials without temperature preference							Trials with temperature preference						
	Concentration prior to avoidance (mg/liter)			Avoidance concentration (mg/liter)			Avoidance of CRC (mg/liter)	Concentration prior to avoidance (mg/liter)			Avoidance concentration (mg/liter)			Avoidance of CRC (mg/liter)
	TRC	FRC	HOCl	TRC	FRC	HOCl		TRC	FRC	HOCl	TRC	FRC	HOCl	
Spotfin shiner														
12	0.12	0.01	0	0.21	0.07	0.03	0.22	0.10	0.02	0.01	0.20	0.12	0.06	0.38
18	0.10	0.02	0.01	0.22	0.13	0.05								
24	0.12	0.01	0	0.21	0.07	0.02	0.20	0.10	0.01	0	0.20	0.08	0.03	0.20
30	0.10	0	0	0.21	0.07	0.02								
Whitetail shiner														
12	0.04	0	0	0.11	0.05	0.02	0.22	0.10	0.02	0.01	0.21	0.11	0.05	0.19
18	0.05	0	0	0.11	0.04	0.02								
24	0.05	0	0	0.11	0.04	0.01	0.20	0.06	0	0	0.11	0.03	0.01	0.20
30	0.11	0	0	0.22	0.03	0.01								

TABLE 6.—*Comparison of laboratory chlorine-avoidance concentrations with continuous and intermittent-exposure toxicity values for several fish species. The range of values reported for some of the avoidance thresholds and toxicity tests is due primarily to different acclimation temperatures (10–30 C) used in these studies. TRC is total residual chlorine; LC50 is the concentration that killed 50% of test fish.*

Species	First TRC avoidance concentration (mg/liter)	TRC toxic concentration (mg/liter)
Catostomus commersoni (white sucker)	0.10–0.21[a]	0.25[b] (LC50, 12 hours); 1.09–0.36[c] (LC50, 48 hours)
Cymatogaster aggregata (shiner perch)	0.18[d]	0.23–0.31[d] (LC50, 1 hour)
Cyprinus carpio (common carp)	0.10–0.21[a]	0.70[b] (LC80, 100 hours); 2.37–1.50[c] (LC50, 48 hours)
Ictalurus punctatus (channel catfish)	0.20–0.41[a]	0.78–0.67[c] (LC50, 48 hours)
Micropterus punctulatus (spotted bass)	0.05–0.20[e]	No data available
Notropis cornutus (common shiner)	0.11–0.21[f]	0.70[b] (100% mortality, 1.3 hours); 0.78–0.45[c] (LC50, 48 hours)
Notropis galacturus (whitetail shiner)	0.11–0.22[g]	No data available
Notropis rubellus (rosyface shiner)	0.05–0.20[e]	0.07 (100% mortality, 3 hours); 0.70 (100% mortality, 1.3 hours)
Notropis spilopterus (spotfin shiner)	0.20–0.38[g]	0.65–0.41[c] (LC50, 48 hours)
Oncorhynchus kisutch (coho salmon)	0.002[d]; 0.05–0.10[a]	0.13–0.21[d] (LC50, 1 hour); 0.16–0.64[h] (LC50, 48–120 hours)
Pimephales notatus (bluntnose minnow)	0.10–0.20[i]	0.11[b] (LC60, 30 days)
Pimephales promelas (flathead minnow)	0.12[j]	0.08–0.19[b] (LC50, 96 hours)
Rhinichthys atratulus (blacknose dace)	0.13[k]	0.70 (100% mortality, 1.3 hours)

[a] Cherry et al. (1979).
[b] Toxicity concentrations from several authors summarized by Mattice and Zittel (1976).
[c] TRC predominantly (>90%) monochloramine intermittently injected in four 40-minute exposures over 24 hours with the LC50 obtained 48 hours thereafter by Seegert et al. (1979).
[d] Value reported as total residual oxidant (TRO) in saltwater study by Stober et al. (1980).
[e] Cherry$_3$ et al. (1977).
[f] Cherry et al. (1978).
[g] This study.
[h] Intermittent treatment of triple 45-minute exposures daily with TRC (LC50 = 0.16) or combined residual chlorine (LC50 = 0.64) by Heath (1977).
[i] Cherry$_2$ et al. (1977).
[j] Larrick et al. (1978).
[k] Fava and Tsai (1976).

tail shiners. In thermal discharge temperatures above 30 C, whitetail shiners first avoided a TRC concentration (0.04 mg/liter) one-third that avoided by spotfin shiners (0.12 mg/liter). Stauffer et al. (1976) found that both species were distributed over broad thermal conditions and that both would inhabit water up to 35 C. The final temperature preferendum for the spotfin shiner, however, was 2–3 C higher than that for whitetail shiner, indicating a higher thermal tolerance (Table 4). The increased sensitivity of the whitetail shiner to chlorine at higher temperatures, however, was not apparent in the laboratory studies; rather, they avoided lower TRC concentrations at 12, 18, and 24 (0.11 mg/liter) than at 30 C (0.22 mg/liter). Reasons for this discrepancy between the laboratory and field data are not readily apparent, although the HOCl content was consistent at the laboratory avoidance concentrations (Table 5). The influence of HOCl content upon avoidance of TRC in the river could not be addressed adequately due to the wide fluctuation in CRC that comprised the TRC. In field concentrations where HOCl levels were adequate to influence avoidance behavior, CRC concentrations also were high enough (≥0.20 mg/liter) to be an influential factor. Whereas avoidance in the laboratory was measured at 0.11 mg/liter TRC (0.01–0.02 mg/liter HOCl), field samples failed to contain any HOCl until TRC concentrations exceeded 0.11 mg/liter and approached 0.18 mg/liter. Therefore, the better water quality conditions in the laboratory at certain periods in the fall (12, 18, 24 C), when tests were run, probably contributed to the apparent discrepancy between field and laboratory data. If the CRC avoidance concentrations (0.20–0.22 mg/liter at 12 and 24 C) generated in the laboratory are compared to field obser-

vations below 30 C, the results between the laboratory and field are quite consistent.

The only unusually high laboratory avoidance concentration was 0.38 mg/liter CRC, occurring when spotfin shiners selected heated water (21 C) after 12 C acclimation. The preference of cold-acclimated spotfin shiners for warm water may override their avoidance responses to CRC. A similar trend was observed by Cherry et al. (1978) for the common shiner. This species avoided the same concentrations of TRC and CRC (0.20 mg/liter) at 12 and 24 C, but when fish acclimated to 12 C were allowed to select a warmer temperature, they avoided CRC only when it reached 0.40 mg/liter. This tendency could have adaptive value, particularly for species that overwinter in the discharge area. Attempts to evaluate this condition were unsuccessful in our field studies because fish were rare or absent in seine hauls when water temperatures were at or below 18 C. Additional seining efforts in other areas of this drainage system suggested that as ambient temperature dropped too low, fish may have aggregated in narrow preferred-temperature bands along the river-effluent "ecotone" and, hence, became unavailable to standard sampling at sites 1–3. This hypothesis requires further study.

A comparison between threshold avoidance concentrations and continuous exposure chlorine toxicity values (as presented by Mattice and Zittel 1976; Seegert et al. 1979; and others) showed that several fish species would avoid concentrations of chlorine before acute toxic effects became apparent (Table 6). Relatively few intermittent-toxicity tests have been conducted, but these more accurately reflect exposure conditions in the thermal effluent under study. Intermittent lethal concentrations causing 50% mortality (LC50) were estimated to be 0.65, 0.59, and 0.41 mg/liter for spotfin shiners (four 40-minute exposures to monochloramine in 24 hours) at 10, 20, and 30 C, respectively, when LC50 determinations were calculated 48 hours later (Seegert et al. 1979). The most frequent avoidance concentration—0.20 mg/liter TRC—established for spotfin shiners in this study indicates this species detected and avoided concentrations at least 50% below those resulting in acute toxic effects. Furthermore, avoidance of chlorine in the laboratory occurred either instantly or within the first few minutes of the 10-minute observation period, whereas lethal effects in bioassays may not become apparent with exposure to these levels for hours or, in some instances, days. Some fish, therefore, may be capable of exploiting potentially lethal conditions for limited periods of time (such as the 40-minute field exposures measured at Glen Lyn).

The lowest field concentration that caused avoidance, 0.15 mg/liter TRC, is not within the suggested range (0.003–0.005 mg/liter TRC for continuous exposure or 0.05 mg/liter for one 30-minute exposure per day) for protection of aquatic organisms (NAS and NAE 1973; Thurston et al. 1979). These criteria, however, were based largely on continuous-exposure toxicity tests of *Gammarus* species, fathead minnows, and salmonids. Organisms can tolerate much higher levels of chlorine under short-term exposure conditions, but the long-term effects of repeated exposures need further investigation (NAS and NAE 1973).

Few studies have examined the avoidance responses of fish to aquatic pollutants in the field. Adult Atlantic salmon (*Salmo salar*) were reported to avoid copper and zinc contamination from a base-metal mine at concentrations of about 16–17 μg/liter (Sprague et al. 1965). Also, Geckler et al. (1976) found that chronic toxicity tests underestimated the safe field concentrations of copper because avoidance was not considered but had a significant effect on fish populations in the stream. Water quality criteria and effluent limitations are intended to protect aquatic life in general. Simply measuring toxicant levels that cause fish (or invertebrate) avoidance may or may not be predictive of safe levels for the majority of species. This information, however, has broadened our understanding of the potential environmental impacts caused by aquatic pollutants, particularly chlorine. Thus, we believe that avoidance data should be considered when water quality criteria and chlorine discharge permit limitations are established for power plants. This study illustrates that avoidance behavior, in general, may be an ecologically significant response by which fish can adjust to harmful conditions, provided areas of refuge are available.

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